The Veterinary Journal 191 (2012) 58–64

Contents lists available at ScienceDirect

The Veterinary Journal

journal homepage: www.elsevier.com/locate/tvjl

# Sinonasal and sino-orbital aspergillosis in 23 cats: Aetiology, clinicopathological features and treatment outcomes

V.R. Barrs [a,*], C. Halliday [b], P. Martin [c], B. Wilson [a], M. Krockenberger [c], M. Gunew [d], S. Bennett [e], E. Koehlmeyer [f], A. Thompson [g], R. Fliegner [h], A. Hocking [i], S. Sleiman [b], C. O'Brien [h], J.A. Beatty [a]

[a] Valentine Charlton Cat Centre, Faculty of Veterinary Science, The University of Sydney, New South Wales 2006, Australia
[b] Centre for Infectious Diseases and Microbiology, Westmead Hospital, Westmead, New South Wales, Australia
[c] Veterinary Pathology Diagnostic Services, Faculty of Veterinary Science, The University of Sydney, New South Wales 2006, Australia
[d] The Cat Clinic, Mt Gravatt, Queensland 4122, Australia
[e] Department of Veterinary Clinical Sciences, Murdoch University, South Street, Murdoch, Western Australia 6150, Australia
[f] Anvet Beenleigh Surgery, Beenleigh, Queensland 4207, Australia
[g] Veterinary Specialist Services, Springwood, Queensland 4217, Australia
[h] University of Melbourne, Veterinary Clinic and Hospital, Werribee, Victoria 3030, Australia
[i] CSIRO Food and Nutritional Sciences, North Ryde, New South Wales 2113, Australia

ARTICLE INFO

*Article history:*
Accepted 2 February 2011

*Keywords:*
Aspergillosis
*Aspergillus* spp.
*Neosartorya* spp.
Cats
Sinonasal
Sino-orbital

ABSTRACT

Aetiology, clinicopathological findings and treatment outcomes were documented in 23 cats (1.5–13 years of age) with sinonasal (SNA, $n = 6$) or sino-orbital (SOA, $n = 17$) aspergillosis. Cases recruited retrospectively and prospectively were included if fungal hyphae were identified on cytological or histological examination and the fungal pathogen was identified by PCR and DNA sequencing (ITS1 or ITS1-5.8S-ITS2 regions, rDNA gene cluster).

Fungal culture was positive in 22/23 cases. In cases of SNA, the fungal pathogen was *Aspergillus fumigatus* ($n = 4$), *Neosartorya fischeri* or *A. lentulus* ($n = 1$) or a non-speciated *Neosartorya* spp. ($n = 1$). In all cases of SOA ($n = 17$), the fungal pathogen was identified as *Neosartorya* spp. Nine cats had brachycephalic conformation. Cats with SNA were more likely to be infected with *A. fumigatus* and had a better prognosis than cats with SOA.



## Introduction

Information about aspergillosis affecting the upper respiratory tract (URT) of cats is restricted to individual case reports and several small case series (Peiffer et al., 1980; Wilkinson et al., 1982; Goodall et al., 1984; Halenda and Reed, 1997; Hamilton et al., 2000; Tomsa et al., 2003; Malik et al., 2004; Whitney et al., 2005; McLellan et al., 2006; Kano et al., 2008; Barachetti et al., 2009; Furrow and Groman, 2009; Karnik et al., 2009; Giordano et al., 2010; Quimby et al., 2010; Smith and Hoffman, 2010). Ten of 25 cats in these reports were Persians or Himalayan Persians, suggesting a possible brachycephalic breed predisposition. Sinonasal aspergillosis (SNA) accounted for approximately half of the reported cases, while the rest had orbital involvement (sino-orbital aspergillosis, SOA). There is limited information on the aetiopathological differences between these two disease presentations, although progression of SNA to SOA has been documented (Hamilton et al., 2000).

The identity of fungal pathogens to species level has been reported in nine affected cats: *Aspergillus fumigatus* ($n = 5$), *A. flavus* ($n = 1$), *A. niger* ($n = 2$) and *A. udagawae* ($n = 1$) (Malik et al., 2004; Whitney et al., 2005; McLellan et al., 2006; Kano et al., 2008; Barachetti et al., 2009; Furrow and Groman, 2009; Smith and Hoffman, 2010; Giordano et al., 2010). However, species identification (*A. udagawae*) was confirmed by molecular studies in only one case; this isolate was initially misidentified as *A. fumigatus* based on phenotypic features (Kano et al., 2008). Members of the *A. fumigatus* complex cannot be identified reliably by phenotypic testing alone (Balajee et al., 2005; Vinh et al., 2009). These findings raise the possibility that pathogens other than *A. fumigatus* could be an underdiagnosed cause of URT aspergillosis in cats.

Treatment of the orbital form of disease is particularly challenging and the prognosis for resolution of infection is generally poor (Hamilton et al., 2000; Kano et al., 2008; Barachetti et al., 2009; Giordano et al., 2010). Few cases have been treated successfully (McLellan et al., 2006; Smith and Hoffman, 2010). The objectives of this study were to document the clinicopathological findings, molecular identity of fungal pathogens and treatment outcomes in cats with URT aspergillosis.

* Corresponding author. Tel.: +61 2 93513437.
*E-mail address:* vanessa.barrs@sydney.edu.au (V.R. Barrs).

1090-0233/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tvjl.2011.02.009

## Materials and methods

### Cases and samples

Retrospective cases were identified from the medical records of the University Veterinary Teaching Hospital, Sydney (UVTHS) and Veterinary Pathology Diagnostic Services (VPDS), from January 1998 to December 2006. Cases were recruited prospectively from January 2007 to December 2009 following an Australia-wide call for cases (Barrs et al., 2007). Inclusion criteria were (1) identification of fungal hyphae on cytological or histological examination of tissue biopsies or sinonasal fungal plaques and (2) molecular identification of the isolate from tissue samples (fresh or formalin-fixed paraffin-embedded tissue) and/or culture material. Cats were classified as having SNA or SOA on the basis of absence (SNA) or presence (SOA) of a retrobulbar mass at initial presentation. Clinical data, tissue samples (fresh and/or formalin-fixed paraffin-embedded tissue) and fungal cultures were collected from each case. Data from postmortem examination were included when available.

### Clinical data

Signalment, history, clinical signs, haematology, biochemistry, retrovirus serology, latex cryptococcal antigen titres (LCAT), agar gel immunodiffusion (AGID) serology for *Aspergillus* spp. antibodies, microbiology, histopathology, treatment and outcome were recorded. Treatment response was categorised as complete remission or treatment failure; complete remission was defined as resolution of all signs $\geqslant$3 months after cessation of therapy; other outcomes were assigned to the treatment failure group. Cats that could not be assessed for treatment response were censored.

### Morphological identification

Samples were cultured at 28 °C and 37 °C on Sabouraud's dextrose agar with added gentamicin and chloramphenicol when bacterial contamination was likely. Where available, isolates identified as *Neosartorya* spp. were retrieved and subcultured on malt extract agar in pairs at 30 °C for 30 days in the dark and examined for cleistothecia (fruiting bodies) at the colony junction. Antifungal susceptibility testing was performed at the Australian Reference Laboratory in Medical Mycology, Adelaide.

### Molecular identification

DNA extraction, PCR amplification of the ITS1 region (fresh and formalin-fixed paraffin-embedded tissue) and/or ITS1-5.8S-ITS2 region (culture material) of the rDNA gene cluster was performed from clinical specimens as described previously using ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) primers (Chen et al., 2002; Lau et al., 2007). Sequence identity was determined using BLAST against the GenBank[1] and Centraalbureau voor Schimmelcultures (CBS)[2] databases.

### Statistical analysis

Statistical analysis was performed using R version 2.6.2. Cats with SNA and SOA were compared with respect to conformation, clinical signs, fungal isolate and response to treatment. Due to the low expected frequencies in some categories, Fisher's Exact Tests were used in preference to $\chi^2$. The fisher.test() function of *R* was used to calculate the *P* value of conditional independence, the conditional Maximum Likelihood Estimate of the odds ratio and the 95% confidence interval of the odds ratio. Significance was ascribed to a *P* value <0.05.

## Results

### Clinical and clinicopathological findings

Twenty-three cases (4 retrospective, 19 prospective) from New South Wales ($n$ = 10), Queensland ($n$ = 9), Victoria ($n$ = 3) and Western Australia ($n$ = 1) met the inclusion criteria. All cats were neutered (13 female, 10 male). The age range was 1.5–13 years (mean 5.3, median 5 years). There were 12 domestic crossbreds (11 domestic short hair, 1 domestic long hair) and 11 pure breeds comprising one Russian Blue, one Cornish Rex and nine cats with brachycephalic conformation (3 Himalayan Persians, 3 Chinchilla Persians, 2 Ragdolls, 1 Exotic Shorthair) (Table 1).

Six cats had SNA and 17 cats had SOA. Lack of orbital involvement was confirmed by advanced diagnostic imaging (CT; $n$ = 5) or surgical exploration of the affected frontal sinus and ipsilateral orbit ($n$ = 1) in all cases of SNA. In cases of SOA, the presence of a retrobulbar mass was confirmed by advanced imaging (computerised tomography or magnetic resonance imaging; $n$ = 11), at surgery or postmortem examination ($n$ = 4) or by histological confirmation of a fungal granuloma where the retrobulbar mass had perforated into the oral cavity ventrally ($n$ = 2). Four of six cats with SNA and 5/17 cats with SOA were brachycephalic; brachycephalic cats were no more likely to have SNA than non-brachycephalic cats. Five of six SNA and 17/17 SOA cases had a history of sneezing and nasal discharge within the preceding 6 months, whereas 8/17 SOA cases had no signs of sinonasal cavity disease at presentation (Table 2). The only significantly different clinical signs between cats with SNA and SOA were exophthalmos ($P < 0.001$) or presence of a mass or ulcer in the pterygopalatine fossa ($P < 0.05$) (Table 2; Fig. 1).

Retrovirus serology and LCAT were negative in all cats tested (FIV: $n$ = 14; FeLV: $n$ = 11; LCAT: $n$ = 9). *Aspergillus* spp. serology (AGID) was positive in 2/2 cats tested (Table 1). Haematological and serum biochemistry findings for nine cats are presented in Table 3. Five cats with SOA and one with SNA were hyperglobulinaemic; these six cats all had *Neosartorya* spp. infections.

### Microbiology

Fungal culture was positive in 22/23 cases. In cases of SNA, the molecular identity of fungal pathogens was *A. fumigatus* ($n$ = 4), *Neosartorya fischeri* or *A. lentulus* ($n$ = 1) and a non-speciated *Neosartorya* spp. ($n$ = 1). Fungal isolates from all 17 cases of SOA were identified as *Neosartorya* spp. by DNA sequencing (Table 1). Fourteen *Neosartorya* spp. were subcultured in pairs. Cleistothecia and ascospores were produced by all isolates from at least one pairing (Fig. 2). No isolates were homothallic. Fourteen of 16 isolates of *A. fumigatus* or *Neosartorya* spp. were resistant to fluconazole (Table 4). Only isolates of *Neosartorya* spp. were resistant to itraconazole (3/13), voriconazole (2/11) and/or posaconazole (1/11). All three isolates of *A. fumigatus* tested were susceptible to voriconazole and posaconazole. No isolates were resistant to amphotericin B.

### Histopathology

Inflammatory infiltrates were lymphocytic ($n$ = 2), histiocytic and eosinophilic ($n$ = 1) or neutrophilic ($n$ = 1) in nasal mucosal biopsies available from 4/6 SNA cases. Biopsies were available from 13/17 SOA cases. Granulomatous ($n$ = 1) or plasmacytic and eosinophilic ($n$ = 1) rhinitis were evident in nasal biopsies from two cases. Retrobulbar ($n$ = 11) and nasopharyngeal ($n$ = 2) masses were characterised by necrosis and well-vascularised granulomatous inflammation. Granulomas contained central areas of coagulative and liquefactive necrosis with abundant periodic acid-Schiff-positive fungal hyphae. Surrounding zones of inflammation comprised epithelioid macrophages interspersed with variable numbers of eosinophils, neutrophils, lymphocytes and plasma cells extending into a peripheral zone of fibrosis.

Complete postmortem examinations were performed in six cats with SOA. All had granulomatous mycotic invasion of the nasal cavities and paranasal sinuses, with variable invasion of the submucosal tissue, invasion of paranasal soft-tissues ipsilateral to the affected orbit and lysis of bone (Fig. 3). Inflammatory lesions effaced the adjacent skeletal muscle and bone in some cases.

Two cats that had surgical exenteration of the right orbit subsequently became blind in the left eye. In one case, there was mycotic involvement of the optic chiasm. In the other case, a retrobulbar mass effaced the left optic nerve. Of nine SOA cases in which ocular

[1] http://www.ncbi.nlm.nih.gov/genbank/.
[2] http://www.cbs.knaw.nl/databases/.